Remarks

The above amendments were made simply to correct typographical, spelling, and grammatical errors, and to correct the page numbers of a document citation on page 6, line 27. As the author, journal title and volume, and year of publication of the document were correctly cited, one skilled in the art would easily have found the correct page numbers. No new matter has been added as a result of these amendments.

Conclusion

The Examiner is invited to contact Applicants' Representatives at the below-listed telephone number, if there are any questions regarding this Preliminary Amendment or if prosecution of this application may be assisted thereby.

Respectfully submitted for
Lawrence P. Wackett et al.
By
Mueting, Raasch & Gebhardt, P.A.
P.O. Box 581415
Minneapolis, MN 55458-1415
Phone: (612)305-1220
Facsimile: (612)305-1228
Customer Number 26813

26813
PATENT TRADEMARK OFFICE

April 1, 2002
Date

By: 
David L. Provence
Reg. No. 43,022
Direct Dial (612)305-1005

APPENDIX A - SPECIFICATION AMENDMENTS
INCLUDING NOTATIONS TO INDICATE CHANGES MADE
Serial No.: 09/898,238
Docket No.: 110.0023 0102

Amendments to the following are indicated by underlining what has been added and bracketing what has been deleted. Additionally, all amendments have been bolded.

In the Specification

The paragraph at page 1, lines 5-7, has been amended as follows:

This invention was made with government support from the United States Department of Agriculture-BARD program, Grant No. **[94-34339-112]** **94-34339-1122**. The government may have certain rights in this invention.

The paragraph at page 1, lines 16-22, has been amended as follows:

Numerous studies on the environmental fate of atrazine have shown that atrazine is a recalcitrant compound that is transformed to CO_2 very slowly, if at all, under aerobic or anaerobic conditions. It has a water solubility of 33 mg/l at 27°C. Its half-life (i.e., time required for half of the original concentration to dissipate) can vary from about 4 weeks to 57 weeks if in soils at low concentration (i.e., less than about 2 parts per million (ppm)). High **[concentration]** **concentrations** of atrazine, such as those occurring in spill sites, have been reported to dissipate even more slowly.

The paragraph at page 2, lines 1-28, has been amended as follows:

There have been numerous reports **[on]** **of** the isolation of *s*-triazine-degrading microorganisms (see, e.g., Behki et al., J. Agric. Food Chem., 34, 746-749 (1986); Behki et al., Appl. Environ. Microbiol., 59, 1955-1959 (1993); Cook, FEMS Microbiol. Rev., 46, 93-116 (1987); Cook et al., J. Agric. Food Chem., 29, 1135-1143 (1981); Erickson et al., Critical Rev. Environ. Cont., 19, 1-13 (1989);

Giardina et al., Agric. Biol. Chem., 44, 2067-2072 (1980); Jessee et al., Appl. Environ. Microbiol., 45, 97-102 (1983); Mandelbaum et al., Appl. Environ. Microbiol., 61, 1451-1457 (1995); Mandelbaum et al., Appl. Environ. Microbiol., 59, 1695-1701 (1993); Mandelbaum et al., Environ. Sci. Technol., 27, 1943-1946 (1993); Radosevich et al., Appl. Environ. Microbiol., 61, 297-302 (1995); and Yanze-Kontchou et al., Appl. Environ. Microbiol., 60, 4297-4302 (1994)). Many of the organisms described, however, failed to mineralize atrazine (see, e.g., Cook, FEMS Microbiol. Rev., 46, 93-116 (1987); and Cook et al., J. Agric. Food Chem., 29, 1135-1143 (1981)). While earlier studies have reported atrazine degradation only by mixed microbial consortia, more recent reports have indicated that several isolated bacterial strains can degrade atrazine. For example, we previously reported the isolation of a pure bacterial culture, identified as *Pseudomonas* sp. strain ADP (Mandelbaum et al., Appl. Environ. Microbiol., 61, 1451-1457 (1995); Mandelbaum et al., Appl. Environ. Microbiol., 59, 1695-1701 (1993); and Mandelbaum et al., Environ. Sci. Technol., 27, 1943-1946 (1993)), which degraded a high concentration of atrazine (>1,000 μg/ml) under growth and non-growth conditions. See also, Radosevich et al., Appl. Environ. Microbiol., 61, 297-302 (1995) and Yanze-Kontchou et al., Appl. Environ. Microbiol., 60, 4297-4302 (1994). *Pseudomonas* sp. strain ADP (Atrazine Degrading *Pseudomonas*) uses atrazine as a sole source of nitrogen for growth. The organism completely mineralizes the *s*-triazine ring of atrazine under aerobic growth conditions. That is, this bacteria is capable of degrading the *s*-triazine ring and mineralizing organic intermediates to inorganic compounds and ions (e.g., CO_2).

The paragraph at page 6, line 21, to page 7, line 2, has been amended as follows:

The 1.9-kb *Ava*I genomic fragment includes the gene that encodes an enzyme that transforms atrazine to hydroxyatrazine, atrazine chlorohydrolase. As used herein, this gene is referred to as "*atz*A", whereas the protein that it encodes is referred to as "AtzA". Hydroxyatrazine formation in the environment was previously thought to result solely from the chemical hydrolysis of atrazine (Armstrong et al., Environ. Sci. Technol., 2, 683-689 (1968); deBruijn et al., Gene, 27, 131-149 (1984); and Nair et al., Environ. Sci. Technol., 26, **[1627-1634]** **2298-2300** (1992)). In contrast to reports that the first step in atrazine degradation by environmental bacteria is dealkylation, this suggests that biological transformation of atrazine to hydroxyatrazine may be widespread in natural systems.

The paragraph beginning at page 25, lines 21-23, has been amended as follows:

[a]Determined by TLC analysis according to procedures described in the materials and methods. Unlabelled desisopropylatrazine and desethylatrazine had R_f **[vales]** **values** of 0.79 and 0.83, respectively.

The paragraph beginning at page 26, lines 16-25, has been amended as follows:

Protein Characterization. Protein subunit sizes were determined by SDS polyacrylamide gel electrophoresis by comparison to known standard proteins, using a Mini-Protean II gel apparatus (Biorad, Hercules, CA). The size of the holoenzyme was determined by gel filtration chromatography on a Superose 6 HR (1.0 x 30.0 cm) column, using an FPLC System (Pharmacia, Uppsala, Sweden). The protein was eluted with 25 mM MOPS buffer (pH 6.9) containing 0.1 M NaCl. Proteins with known molecular weights were used as chromatography standards.

Isoelectric point determinations were done using a **[Pharamacia]** **Pharmacia** Phast-Gel System and **[Pharamacia]** **Pharmacia** IEF 3-9 media. A **[Pharamacia]** **Pharmacia** broad-range pI calibration kit was used for standards.

The paragraph at page 35, lines 14-28, has been amended as follows:

Several lines of evidence support the conclusion that the designated ORF constitutes the atrazine chlorohydrolase gene: 1) *E.coli* transformed with pMD4, gained the ability to degrade atrazine as demonstrated by clearing zones surrounding colonies on solid media containing crystalline atrazine, 2) the dechlorination activity was abolished by transposon *Tn*5 insertions specifically within the 1.9-kb *Ava*I fragment and the Tn5 insertion was located within the ORF, 3) there is also significant homology between the *atz*A ORF (40.987% identity over 484 amino acid residues) and a protein from *Rhodococcus corallinus* NRRL B-15444R which possesses an analogous catalytic activity, a triazine hydrolase which is responsible for the deamination of melamine (2,4,6-triamino-1,3,5-triazine) and dechlorination of deethylsimazine. While no typical *E. coli* -10 sequence was seen preceding the predicted start of *Atz*A, a potential *Pseudomonas* ribosome binding site was found 11 base pairs upstream of the ATG (V. **[Shigler]** **Shingler** et al., J. Bacteriol., 174, 711-724 (1992)). This is interesting given the fact that atzA was expressed in *E. coli*.